# PRODUCTION OF PROTEINS IN GLUTAMINE-FREE CELL CULTURE MEDIA

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 13/945,531, filed Jul. 18, 2013, now abandoned, which is a continuation of U.S. application Ser. No. 12/852,377, filed Aug. 6, 2010, now U.S. Pat. No. 8,512,983, issued Aug. 20, 2013, which claims priority under 35 USC §119(e) and the benefit of U.S. Provisional application No. 61/232,889, filed Aug. 11, 2009, the contents of which are incorporated herein by reference in their entireties.

## BACKGROUND OF THE INVENTION

Mammalian cells have become the dominant system for the production of mammalian proteins for clinical applications, primarily due to their ability to produce properly folded and assembled heterologous proteins, and their capacity for post-translational modifications. It is conventional to have glutamine in cell culture media during recombinant production of heterologous proteins, including antibodies. L-glutamine is an essential amino acid, which is considered the primary energy and nitrogen sources for cells in culture. Most commercially available media are formulated with free L-glutamine which is either included in the basal formula or added to liquid media formulations at the time of use. Thus, all mammalian cell culture media contain glutamine except those for glutamine synthetase transfected cell lines, such as GS NS0 and GS CHO cell lines, where the cells themselves produce the glutamine needed for growth. Glutamine is widely used at various concentrations typically from 1 to 20 mM in base media and much higher concentration in feeds for fed-batch process. For example, the concentration of L-glutamine is 0.5 mM in Ames' Medium and 10 mM in MCDP Media 131. DMEM/Ham's Nutrient Mixture F-12 (50:50) is often used as a starting formulation for proprietary media used with Chinese Hamster Ovary (CHO) cells. L-glutamine in DMEM/Ham's Nutrient Mixture F-12 is 2.5 mM. L-glutamine concentration in Serum-Free/Protein Free Hybridoma Medium is 2.7 mM. L-glutamine in DMEM, GMEM, IMDM and H-Y medium is 4 mM, of which IMDM is often used as a starting formulation for proprietary hybridoma cell culture media. It is generally held that hybridoma cells grow better in concentrations of L-glutamine that are above the average levels found in media. (Dennis R. Conrad, Glutamine in Cell Culture, Sigma-Aldrich Media Expert)

It was shown that glutamine is the main source of ammonia accumulated in cell culture (see review by Markus Schneider, et. al. 1996, *Journal of Biotechnology* 46:161-185). Thus, lowering glutamine in cell culture media significantly reduced the accumulation of $NH_4^+$ level, resulting in lower cytotoxicity (see Markus Schneider, et. al. 1996, supra). Reduced $NH_4^+$ cytotoxicity resulted in higher cell viability, thus extended culture longevity. Based on an estimated glutamine consumption study using CHO cells, it was suggested that cells may consume glutamine at a rate of 0.3-0.4 mM per day (Miller, et. al. 1988, Biotechnol. Bioeng. 32: 947-965). Altamirano et al. (2001, *J. Biotechnol.* 110:171-9) studied the effect of glutamine replacement by glutamate and the balance between glutamate and glucose metabolism on the redistribution of CHO cells producing recombinant human tissue plasminogen activator (rhut-PA). When glutamine was replaced with glutamate and balanced with glucose catabolism (carbon and nitrogen ratio, C/N ratio), cell metabolism was found redistributed and forced to utilize carbon and energy source more favorably to production of rhut-PA. It was also reported that CHO cells in adherent cultures can grow in the absence of added glutamine due to endogenous glutamine synthetase activity that allowed cells to synthesize glutamine from glutamic acid in the medium (Sanfeliu and Stephanopoulos, 1999, *Biotechnol. Bioeng.* 64:46-53). However, compared to control cultures in glutamine-containing media, the cell growth rate in glutamine-free media was slower with an increased fraction of cells distributed in the G0/G1 phase. The depletion of both glutamine and glutamic acid did cause cell death.



## SUMMARY OF THE INVENTION

The present invention is based, at least in part, on the unexpected finding that not only can recombinant proteins be produced in a mammalian host cell using a glutamine-free production medium without any significant adverse effect, in fact the use of a glutamine-free medium in the production phase significantly increases cell viability, culture longevity, specific productivity and/or the final recombinant protein titer.

The present invention is also based on the unexpected finding that the addition of asparagine to a glutamine-free production medium can further enhance the cell viability, culture longevity, specific productivity and/or the final recombinant protein titer in a mammalian host cell using a glutamine-free production medium without any significant adverse effect.

In one aspect, the invention concerns a process for producing a polypeptide in a mammalian host cell expressing said polypeptide, comprising culturing the mammalian host cell in a production phase of the culture in a glutamine-free production culture medium supplemented with asparagine.

In one embodiment, the mammalian host cell is a Chinese Hamster Ovary (CHO) cell.

In another embodiment, the mammalian host cell is a $dhfr^-$ CHO cell.

In yet another embodiment, the production medium is serum-free.

In a further embodiment, the production culture medium comprises one or more ingredients selected from the group consisting of

1) an energy source;
2) essential amino acids;
3) vitamins;
4) free fatty acids; and
5) trace elements.

In a still further embodiment, wherein the production culture medium additionally comprises one or more ingredients selected from the group consisting of:

1) hormones and other growth factors;
2) salts and buffers; and
3) nucleosides.

In all embodiments, the production phase may, for example, be a batch or fed batch culture phase.

In all embodiments, the process may further comprise the step of isolating said polypeptide.

In a further embodiment, isolation may be followed by determining one or more of cell viability, culture longevity, specific productivity and final recombinant protein titer following isolation.

In a still further embodiment, at least one of the cell viability, culture longevity, specific productivity and final recombinant protein titer is increased relative to the same